orin fused to the lumenal aspect of TGN38 to measure pH of the TGN (pH_{TGN}). Immunofluorescence showed that H/K-ATPase was present in the plasma membrane and the TGN of H/Kα,β cells. When the TGN was alkalinized (after a brief pulse of acetate, 30 mM), reacidification was completely blocked by bafilomycin (inhibitor of the H+ v-ATPase) in mock cells, but full block of reacidification in H/Kα,β cells required both bafilomycin and SCH28080 (inhibitor of H/K-ATPase). SCH28080 had no effect on pH_{TGN} in mock cells but caused pH_{TGN} to alkalinize slowly (2×10^{-4} pH/s) from pH 6.4 by 0.15 pH units in H/Kα,β cells; subsequent addition of bafilomycin caused pH_{TGN} to alkalinize 8–10 times more rapidly than during SCH28080 treatment, up to a new steady-state pH 7.0–7.5, similar to cytosol. Bafilomycin caused similarly rapid rates of alkalinization of pH_{TGN} in mock and H/Kα,β cells, indicating that the TGN (like the Golgi; see Wu et al. 2001. *J. Biol. Chem.* 276:33027–33035) had a large H^+ permeability. Thus, the TGN has large permeabilities to K^+ and Cl^-, which provide the ions required for H/K-ATPase function, and also to H^+, which constantly leak H^+ accumulated by both H^+ v-ATPase and H/K-ATPase. Model calculations indicated that pH_{TGN} was only 0.15 pH lower in H/Kα,β cells than in mock cells because the high H^+ permeability of the Golgi (10^{-3} cm/s) demands a large number (10,000) of H^+ v-ATPases to be permanently present in the TGN, making the effects of only 1,000–1,250 H/K-ATPases in transit to the plasma membrane relatively minor. Our data indicate that the TGN has large numbers of H^+ v-ATPases that constantly pump "against" a large H^+ permeability so that the smaller numbers of other H^+ pumps or leaks trafficking through the TGN on their way to the plasma membrane have minimal effect on pH_{TGN}. This H^+ pump-leak buffering mechanism provides an inefficient, but effective system for assuring that the TGN acidity will be consistent across all cell types. (Supported by NIH DK51799 and NSF MCB9983342.)

28. Intracellular Trafficking of the Human Reduced Folate Carrier Is Mediated by the Microtubular Cytoskeleton JONATHAN S. MARCHANT,[1] VEEDAMALI S. SUBRAMANIAN,[2,3,4] IAN PARKER,[1] and HAMID M. SAID,[2,3,4] [1]*Departments of Neurobiology and Behavior,* [2]*Medicine, and* [3]*Physiology/Biophysics, University of California, Irvine, CA; and* [4]*Veterans Affairs Medical Center, Long Beach, CA* (Sponsor: R. Josephson)

The major cellular pathway for uptake of the vitamin folic acid is via a plasma membrane carrier protein known as the reduced folate carrier. We have investigated the mechanisms that control the intracellular trafficking and plasma membrane targeting of the human reduced folate carrier (hRFC) by using confocal microscopy to monitor the cellular distribution of an hRFC fusion protein tagged with the green fluorescent protein (hRFC-EGFP). Transient expression of hRFC-EGFP in a variety of epithelial cell lines (MDCK, HuTu-80, HEK293, and Caco-2 cells) resulted in strong expression of the fusion protein at the cell membrane, assessed by colocalization with the plasma membrane marker FM4-64 (~65% overlap of fluorescence signals after 40 h). Incubation of cells with low concentrations of nocodazole (500 nM for 10 h) attenuated cell surface expression of hRFC-EGFP, whereas treatment with cytochalasin D (500 nM for 10 h) had little effect. Video-rate confocal imaging of a HuTu-80 cell line stably transfected with hRFC-EGFP permitted resolution of the motion of hRFC-containing vesicles. At 37°C, individual vesicles exhibited rapid linear motions (average velocity ~1.9 μm/s) toward as well as away from the cell membrane, interspersed with periods of low motility. Short term incubation with nocodazole (10 μM, <5 min) or colchicine (10 μM, <10 min) inhibited the rapid linear vesicular movements, whereas γ-lumicolchicine (50 μM) and cytochalasin D (10 μM) were ineffective. These data underscore previous evidence (Subramanian et al. 2001. *Am. J. Physiol.* 281: G1477–G1486) that implicate a crucial role for microtubules, but not microfilaments, in the transport of hRFC to the cell surface. (Supported by the Department of Veterans Affairs and NIH grants DK-56061, DK-58057, and GM-48071.)

29. Molecular Determinants for Plasma Membrane Sorting of the Renal Sulfate Transporter Sat-1 DANIEL MARKOVICH and RALF R. REGEER, *Department of Physiology and Pharmacology, School of Biomedical Sciences, University of Queensland, Brisbane, Queensland, Australia*

Sulfate is an essential anion required for bone/cartilage formation, proteoglycan synthesis, and cellular metabolism. Body sulfate homeostasis is primarily maintained through renal tubular mechanisms. The sulfate anion transporter (sat-1) encodes a 703–amino acid protein (75.4 kD) with 12 putative transmembrane domains, which mediates sulfate/chloride/bicarbonate exchange across the basolateral membrane of the renal tubule. To identify the molecular determinant(s) responsible for this basolateral expression pattern in vivo, deletion mutants were made within the intracellular COOH-terminal tail of the rat sat-1 (rsat-1) protein, which were fused to the enhanced green fluorescence protein. These constructs were transiently transfected

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into Mardin-Darby Canine Kidney (MDCK) cells in which the wild-type rsat-1 was sorted exclusively to the basolateral membrane. The removal of the last 30 amino acids of the COOH-terminal tail showed intracellular expression, suggesting the presence of a sorting motif within the last 30 amino acids of this protein. The removal of the PDZ domain (SAL) located at the last three residues on the COOH terminus of rsat-1, had no effect on the plasma membrane sorting of the protein, suggesting that PDZ-binding proteins may not be required for sat-1 basolateral sorting. Sequence analysis of the last 30 amino acid residues of rsat-1 identified a dileucine motif at position $L_{677}L_{678}$ as a putative targeting signal. Site-directed mutagenesis of this dileucine motif (to alanines) led to a loss of basolateral expression, suggesting that this motif was necessary for basolateral trafficking of the rsat-1 protein. The intracellular compartment where this mutant is accumulated is presently being investigated. (Supported by an Australian National Health and Medical Research Council grant to D. Markovich.)

30. Synaptic Targeting of N-type Calcium Channels in Hippocampal Neurons ANTON MAXIMOV and ILYA BEZPROZVANNY, *Department of Physiology, University of Texas Southwestern Medical Center at Dallas, Dallas, TX*

N-type calcium (Ca^{2+}) channels play a critical role in synaptic function, but the mechanisms responsible for their targeting in neurons are poorly understood. N-type channels are formed by α_{1B} pore–forming subunit associated with β and α2δ auxiliary subunits. By expressing epitope-tagged recombinant α_{1B} subunits in rat hippocampal neuronal cultures, here we demonstrate that synaptic targeting of N-type channels depends on neuronal contacts and synapse formation. We further establish that the COOH-terminal 163 amino acids (2177–2339) of $\alpha_{1B\text{-}1}$ ($Ca_V2.2a$) splice-variant contain sequences, which are both necessary and sufficient for synaptic targeting. By site-directed mutagenesis we demonstrate that Mint1-PDZ and CASK-SH3 binding motifs located within this region of α_{1B} subunit (Maximov et al. 1999. *J. Biol. Chem.* 274:24453–24456) act as redundant synaptic targeting signals. We further show that the recombinant modular adaptor proteins Mint1 and CASK colocalize with N-type channels in synapses. We found that $\alpha_{1B\text{-}2}$ ($Ca_V2.2b$) splice variant is restricted to soma and dendrites and postulated that "somatodendritic" and "axonal/synaptic" isoforms of N-type channels are generated via alternative splicing of α_{1B} carboxy termini. These data lead us to propose that during synaptogenesis $\alpha_{1B\text{-}1}$ ($Ca_V2.2a$) splice-variant of N-type Ca^{2+} channels pore-forming subunit recruited to synaptic locations by means of interactions with modular adaptor proteins Mint1 and CASK. Our results provide a novel insight into molecular mechanisms responsible for targeting of Ca^{2+} channels and other synaptic proteins in neurons. (Supported by the Robert A. Welch Foundation and NIH R01 NS39552.)

31. N287Y, a Mutation in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Gives Rise to Clinical Disease by Enhancing Endocytic Retrieval JOHN A. PICCIANO, MARK S. SILVIS, CAROL BERTRAND, ROBERT J. BRIDGES, and NEIL A. BRADBURY, *Cystic Fibrosis Research Centre, Department of Cell Biology and Physiology, University of Pittsburgh School of Medicine, Pittsburgh, PA* (Sponsor: Kirk L. Hamilton)

Mutations in the primary sequence of CFTR give rise to the characteristic phenotype of the genetic disease cystic fibrosis (CF). Such phenotype includes chronic respiratory infections, impaired sweat electrolyte levels and, in severe cases, malfunction of the exocrine pancreas. The most common mutation in CFTR, accounting for ~70% of all mutant alleles is the loss of a phenylalanine residue at position 508 (ΔF508 CFTR). Mechanistically, ΔF508 CFTR results in a protein that folds inappropriately and fails to exit the endoplasmic reticulum. 2,000 mutations have now been described in the CFTR gene, yet the molecular bases for most of these mutations are not known. N287Y, a clinical mutation in the second intracellular loop of CFTR, gives rise to patients with pancreatic sufficiency, mild airway disease, and elevated sweat electrolytes. We, and others, have shown that critical tyrosine-based motifs are important for the correct endocytic trafficking of CFTR. We hypothesized that the N287Y mutations results in the generation of a novel endocytic signal, causing CFTR to be removed prematurely from the plasma membrane. We have stably expressed both wild-type (wt) and N287Y CFTR in Hek293 cells. No difference in total cellular CFTR was observed between the two cell lines. However, N287Y CFTR was present in the plasma membrane at a level ~50% of that observed for wt CFTR. In addition to cell surface labeling, CFTR is also present in early endosomes (EE). Significantly more N287Y CFTR was present in EE compared with wt, suggesting that the reduction in cell surface signal was due to an increase in endocytic retrieval. Indeed, N287Y CFTR was endocytosed at twice the rate of wt CFTR. Whole-cell patch clamp analysis revealed that CFTR activity was also significantly reduced in N287Y CFTR, showing a physiological consequence of increased endocytosis. In summary, the N287Y mutation reveals a novel class of CFTR mutation, whose primary